

CLAIMS:

1. A composition comprising a purified farnesyl:protein transferase enzyme, characterized as follows:

- (a) capable of catalyzing the transfer of farnesol to a protein or peptide having a farnesyl acceptor moiety;
- (b) capable of binding to an affinity chromatography medium comprised of TKCVIM coupled to a suitable matrix;
- (c) exhibiting a molecular weight of between about 70,000 kDa and about 100,000 kDa upon gel filtration chromatography, and comprised of two different subunits, each exhibiting a molecular weight of approximately 45,000 kDa to 50,000 kDa upon SDS-PAGE; and
- (d) having a farnesyl transferase activity that is capable of being inhibited by TKCVIM; CVIM; or KKSKTKCVIM.

2. The composition of claim 1, further defined as exhibiting a farnesyl transferase specific activity of between about 5 and about 600,000 units/mg protein.

3. The composition of claim 2, further defined as exhibiting a farnesyl transferase specific activity of between about 500 and about 600,000 units/mg protein.

4. The composition of claim 1, wherein said farnesyl transferase enzyme is purified by a process which includes the steps of:

- (a) preparing a cellular extract which includes the enzyme;
- (b) subjecting the extract to affinity chromatography on an affinity chromatography medium to bind the enzyme thereto, the medium comprised of a farnesyl transferase binding peptide coupled to a suitable matrix;
- (c) washing the medium to remove impurities; and

(d) eluting the enzyme from the washed medium.

5. The composition of claim 4, wherein the farnesyl transferase binding peptide comprises a peptide of at least 4 amino acids in length and including a carboxy terminal sequence of -C-A-A-X, wherein:

5 C = cysteine;

A = an aliphatic or hydroxy amino acid; and

X = any amino acid

6. The composition of claim 5, wherein the farnesyl transferase binding peptide includes a carboxy terminal sequence of -C-V-I-M, -C-S-I-M or -C-A-I-M.

10 7. The composition of claim 6, wherein the farnesyl transferase binding peptide comprises T-K-C-V-I-M.

8. The composition of claim 1, wherein the farnesyl transferase enzyme is prepared by recombinant means.

9. A method of preparing a farnesyl transferase enzyme, comprising the steps of:

15 (a) preparing a cellular extract which includes the enzyme;

(b) subjecting the extract to affinity chromatography on an affinity chromatography medium to bind the enzyme thereto, the medium comprised of a farnesyl transferase binding peptide coupled to a suitable matrix;

20 (c) washing the medium to remove impurities; and

(d) eluting the enzyme from the washed medium.

10. The method of claim 9, wherein the farnesyl transferase binding peptide comprises a peptide of at least 4 amino acids in length and including a carboxy terminal sequence of -C-A-A-X, wherein:

C = cysteine;

A = an aliphatic or hydroxy amino acid; and

X = any amino acid

11. The method of claim 10, wherein the farnesyl transferase binding peptide includes
5 a carboxy terminal sequence of -C-V-I-M, -C-S-I-M or -C-A-I-M.

12. The method of claim 10, wherein the farnesyl transferase binding peptide is
biotinylated.

13. The method of claim 11, wherein the farnesyl transferase binding peptide
comprises T-K-C-V-I-M.

10 14. A method for assaying for the presence of farnesyl transferase activity in a
composition comprising determining the ability of said composition to catalyze the
transfer of farnesol to a farnesyl acceptor protein or peptide.

15. The method of claim 14, wherein said farnesol is transferred from farnesyl
pyrophosphate.

15 16. The method of claim 15, wherein said farnesyl pyrophosphate contains a label on
the farnesyl moiety.

17. The method of claim 14, wherein said farnesyl acceptor protein or peptide
comprises a carboxy terminal sequence of -C-A-A-X, wherein:

C = cysteine;

20 A = an aliphatic or hydroxy amino acid; and

X = any amino acid

18. The method of claim 17, wherein said farnesyl acceptor protein or peptide
comprises a p21^{ras} protein.

19. The method of claim 17, wherein said farnesyl acceptor protein or peptide comprises a peptide of at least 4 amino acids in length.

20. The method of claim 19, wherein the farnesyl acceptor protein or peptide comprises CVIM; KKSSTKCVIN; TKCVIM; RASNRSCAIM; TQSPQNCSIM; CIIM; CVVN; or CVLS.

21. A farnesyl transferase inhibitor comprising a peptide, or protein other than a p21^{ras} protein, lamin a, lamin b, or yeast mating factor a, said peptide or protein having a farnesyl acceptor or inhibitor sequence within its structure and capable of inhibiting the farnesylation of p21^{ras} by farnesyl transferase.

22. The inhibitor claim 21, wherein the farnesyl acceptor or inhibitor sequence is further defined as a farnesyl acceptor amino acid sequence which includes the amino acids CAAX, wherein:

C = cysteine;

A = an aliphatic or hydroxy amino acid; and

X = any amino acid

23. The inhibitor of claim 22, wherein the farnesyl acceptor or inhibitor amino acid sequence is positioned at the carboxy terminus of the protein or peptide.

24. The inhibitor of claim 23, further defined as peptide of from four to 10 amino acids in length.

25. The inhibitor of claim 24, further defined as a peptide incorporating one of the following peptide sequences at its carboxy terminus: CVIM; KKSSTKCVIM; TKCVIM; RASNRSCAIM; TQSPQNCSIM; CIIM; CVVM; CVLS; CVLM; CAIM; CSIM; CCVQ; CIIC; CIIS; CVIS; CVLS; CVIA; CVIL; CLIL; CLLL; CTVA; CVAM; DKIM; CLIM; CVLM; CFIM; CVFM; CVIF; CEIM; CGIM; CPIM; CVYM; CVTM; CVPM; CVSM; CVIF; CVIV; CVIP; or CVIL.

26. The inhibitor of claim 24, further defined as a tetrapeptide.

27. The inhibitor of claim 25, further defined as one of the following peptides:
CVIM; CIIM; CVVM; CVLS; CVLM; CAIM; CSIM; CCVQ; CIIC; CIIS; CVIS; CVLS;
CVIA; CVIL; CLIL; CLLL; CTVA; CVAM; CKIM; CLIM; CVLM; CFIM; CVFM;
5 CVIF; CEIM; CGIM; CPIM; CVYM; CVTM; CVPM; CVSM; CVIF; CVIV; CVIP; or
CVII.

28. The inhibitor of claim 25, further defined as a peptide having a sequence which
consists essentially of one of the specified peptide sequences.

29. The inhibitor of claim 24 wherein the peptide is modified by biotinylation,
10 esterification, acylation, or alkylation.

30. The inhibitor of claim 23, further defined as a pure inhibitor.

31. The inhibitor of claim 30, further defined as a peptide comprising the structure –
C-A1-A2-X, wherein C = cysteine, A1 – any aliphatic, aromatic or hydroxy amino acid;
A2 = any aromatic amino acid or amino acid modified to incorporate one or more
15 aromatic moieties; and X = any amino acid.

32. The inhibitor of claim 31, further defined as the tetrapeptide CVFM.

33. The inhibitor of claim 31, wherein the aromatic moiety of the A2 amino acid is
modified to include a fluoro, chloro, or nitro group.

34. The inhibitor of claim 33, wherein the A2 amino acid comprises
20 parachlorophenylalanine.

35. The inhibitor of claim 31, wherein the A2 amino acid comprises a naphthyl ring.

36. The method of claim 31, wherein the A2 amino acid comprises phenylalanine,
tyrosine or tryptophan.

37. A method for determining the ability of a candidate substance to inhibit a farnesyl
25 transferase enzyme, comprising the steps of:

- (a) obtaining an enzyme composition comprising a farnesyl transferase enzyme that is capable of transferring a farnesyl moiety to a farnesyl acceptor substance;
- (b) admixing a candidate substance with the enzyme composition; and
- 5 (c) determining the ability of the farnesyl transferase enzyme to transfer a farnesyl moiety to a farnesyl acceptor substrate in the presence of the candidate substance.

38. The method of claim 37, wherein the farnesyl transferase composition comprises the composition of claim 1.

10 39. The method of claim 37, wherein the farnesyl acceptor substrate comprises a p21^{ras}, or any peptide containing a cysteine at the fourth position from the carboxyl terminus.

15 40. The method of claim 37, wherein step (c) comprises determining the ability of the candidate substance to inhibit the transfer of farnesyl from farnesyl pyrophosphate to the acceptor substrate.

41. The method of claim 37, wherein the farnesyl moiety is labeled.

42. The method of claim 41, wherein the farnesyl moiety is radiolabeled.

20 43. A method of inhibiting a farnesyl transferase enzyme comprising subjecting the enzyme to an effective concentration of a farnesyl transferase inhibitor in accordance with claim 21, or a candidate substance identified in accordance with the method of claim 29 to be such an inhibitor.

25 44. A method of inhibiting the attachment of a farnesyl moiety to a *ras* protein in malignant cells comprising subjecting said cells to an effective concentration of a farnesyl transferase inhibitor in accordance with claim 21, or a candidate substance identified in accordance with the method of claim 29 to be such an inhibitor.

45. A DNA segment encoding the α or β subunit of farnesyl:protein transferase.
46. The DNA segment of claim 45, further defined as encoding the α subunit.
47. The DNA segment of claim 45, further defined as encoding the β subunit.
48. A recombinant vector comprising the DNA segment of claim 45.
- 5 49. The recombinant vector of claim 48, further defined as comprising a DNA segment encoding the α subunit of farnesyl protein transferase.
50. The recombinant vector of claim 48, further defined as comprising a DNA segment encoding the β subunit of farnesyl protein transferase.